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THE AGGREGATION PHEROMONE OF *Diorhabda elongata*, A BIOLOGICAL CONTROL AGENT OF SALTCEDAR (*Tamarix* SPP.): IDENTIFICATION OF TWO BEHAVIORALLY ACTIVE COMPONENTS[1]

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Abstract—The leaf beetle *Diorhabda elongata* Brullé (Coleoptera: Chrysomelidae) has been introduced as a biological control agent for saltcedars, *Tamarix* spp., an exotic, invasive weedy tree in the western United State. Gas chromatographic (GC) analysis of volatiles collected from feeding male or female beetles, or saltcedar foliage alone, showed two components produced almost exclusively by males. These compounds elicited responses from antennae of male and female beetles in GC-electroantennographic detection (EAD) analyses. The compounds were identified as (2*E*,4*Z*)-2,4-heptadienal (**1**) and (2*E*,4*Z*)-2,4-heptadien-1-ol (**2**) by GC-mass spectrometry (MS), and confirmed with authentic standards. The two compounds were also detected at trace levels from feeding females and foliage controls, but the amounts from feeding males were 8–40 times higher, typically 55–125 ng per day per male. The amounts of **1** and **2** in collections from females did not differ significantly from amounts collected from control foliage. In field trials, **2** as a single component was as attractive as a 1:1 blend of **1** and **2**. Compound **1** as a single component was more attractive than controls, but much less attractive than **2** or the blend. Males and females

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[1]Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

were attracted in about equal numbers, indicating that this is an aggregation pheromone.

Key Words—*Diorhabda elongata*, Coleoptera, Chrysomelidae, aggregation pheromone, saltcedar, *Tamarix ramosissima*, (2*E*,4*Z*)-2,4-heptadienal, (2*E*,4*Z*)-2,4-heptadien-1-ol, biological control.

INTRODUCTION

Saltcedar (*Tamarix* spp.) is an exotic invasive weedy tree causing up to $285,000,000 of damage per year in water loss due to transpiration and flood damage due to stream channel alteration (Zavaleta, 2000). The invasion of *Tamarix* into riparian areas in the western United States has also caused ecosystem damage that is more difficult to quantify, such as decreased habitat quality for wildlife and native plant species, increased soil salinity, and increased fire risk (Deloach et al., 2000). Efforts to control saltcedar through conventional means such as herbicides or physical removal have been expensive and of limited success. For this reason, a multistate biological control program was initiated in the 1980's (Deloach, 1989). Currently, the only approved and released biological control agent is the leaf beetle *Diorhabda elongata* Brullé (Coleoptera: Chrysomelidae). A release made at Lovelock, Nevada, during 2001 has become particularly well established.

The beetles overwinter as adults in leaf litter and emerge in the spring (typically in May at Lovelock, NV) when saltcedar starts to produce new foliage. Eggs are laid on the foliage. The larvae feed on the leaves, and then pupate in the soil. There are two generations per year at Lovelock, with new adults emerging in July and again in August. Adults from the second generation enter overwintering diapause (DWB, unpublished; Deloach et al., 2004).

One important need in a biological control program is to be able to monitor the beetle populations accurately in the field, and a tool based on the pheromone of the beetles could serve this need. A pheromone-based monitoring system could be useful for studying population attributes such as dispersal rate and distance, local survival, abundance relative to habitat properties, and times of adult activity during the year. Such information can be difficult to obtain with standard sampling methods, especially when the population is sparse. Even in areas with high population density and heavy defoliation, pheromone-based monitoring in surrounding areas could yield information on the behavior of dispersing beetles.

Only a small number of chrysomelid beetle pheromones have been identified from the more than 1,400 chrysomelid species known from North America north of Mexico (Arnett, 1993), but both male- and female-produced long-range pheromones exist within the family. The pheromones are chemically diverse and can consist of either single compounds or blends. The first chrysomelid pheromone

to be chemically identified was that of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Guss et al., 1982). This pheromone is emitted by female beetles and attracts only males. Chemically related, female-produced sex pheromones were subsequently defined for eight *Diabrotica* species and subspecies (summarized by Krysan et al., 1989). These pheromones all consist of methyl-branched esters or ketones.

In contrast, recent studies of chrysomelid chemical communication systems have found only male-produced aggregation pheromones. For example, six male-specific sesquiterpenes were identified from the flea beetle *Phyllotreta cruciferae* Goeze (Bartelt et al., 2001), and a blend of the synthetic compounds was shown to be attractive under field conditions both in Canada and Hungary (J. Soroka, M. Tóth, and R. Bartelt, unpublished). Male-produced aggregation pheromones have been subsequently reported from two other chrysomelid species, the cereal leaf beetle, *Oulema melanopus L.* (Cossé et al., 2002, Rao et al., 2003), and the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Dickens et al., 2002). Chemically, the latter pheromones are unusual hydroxy ketones. In addition, male-produced sesquiterpenes that elicit antennal responses have been identified in three *Aphthona* species (Bartelt et al., 2001)

In the present study we report the identification of two male-produced components of the aggregation pheromone in *D. elongata*.

METHODS AND MATERIALS

Insects. D. elongata adults used in this study were obtained from a colony maintained at the USDA-ARS Exotic and Invasive Weed Research Unit, Albany, CA, USA. The beetles originated from sites near Fukang, in the Xinjiang province of northwestern China (DeLoach et al., 2003, Lewis et al., 2003). Upon arrival in Peoria, IL, adult beetles were kept at 25°C under a 17L:7D h photoperiod. *Tamarix ramosissima* Ledebour (Tamaricaceae) was grown year round in a greenhouse facility at NCAUR to provide food for the beetles.

Volatiles Collections. Initial volatiles collections were made to demonstrate and identify the pheromone, followed by additional collections to quantitate the pheromone emissions.

Beetles (7 to 10 d old) were sexed by inspection of the ventral side of the abdominal tip. A dark internal spot is visible through a sternite in females, and males have a v-shaped indentation at the apex of the last abdominal segment. Volatiles collections were made from individuals and groups of 10 male or female beetles feeding on saltcedar foliage, and from foliage alone. The beetles were placed into a horizontal glass tube (5 cm ID $\times$ 20 cm in length) containing a piece of saltcedar foliage (approx. 15 cm long) with the cut end in a small water vial. A Teflon seal in the cap of the vial kept the water from spilling. Collector equipment

and general volatiles collection procedures were reported earlier (Cossé et al., 2002).

Collections were made in an incubator (25°C, 17L:7D) and volatiles were collected on Super-Q traps (80–100 mesh, Alltech, Deerfield, IL) with an airflow-rate of 100 ml/min. Foliage was replaced daily, and any dead beetles in the collectors were replaced with fresh ones. Collected volatiles were eluted from the traps into vials using methylene chloride (300 μl) every 2–3 d. 1-Octanol (10 μl of a hexane solution containing 25 ng/μl) was added to each vial as a quantitative internal standard. Early in the project, trap breakthrough was evaluated by placing a second Super-Q trap in series with the first. Only compounds of six or fewer carbons were detected in the breakthrough trap.

Electrophysiology. Coupled gas chromatographic-electroantennographic (GC-EAD) analyses were made by methods and equipment generally described by Cossé and Bartelt (2000). GC-EAD connections were made by inserting a glass pipette silver-grounding electrode into the back of an excised beetle head. A second glass pipette silver-recording probe was placed in contact with the distal end of one antenna. Both pipettes were filled with Beadle-Ephrussi saline (Ephrussi and Beadle, 1936).

Instrumentation. Extracts were analyzed by GC with flame-ionization detection (GC-FID) and coupled GC-mass spectrometry (GC-MS). Samples were injected splitless using Hewlett Packard 6890 (Palo Alto, CA) instruments fitted with 30 meter DB-1 or DB-5 capillary columns (0.25 mm I.D., 0.25 μm or 1.0 μm film thickness, J&W Scientific, Folsom, CA). Temperature programs were from 50°C to 275°C at 10°C per min. Inlet temperatures were maintained at 250°C and GC-EAD effluent interface from the post-column splitter was kept at 275°C. Mass spectrometry was performed using an HP 5973 instrument (electron impact, 70 eV). The Wiley mass spectral library, with 275,821 spectra, was available on the MS data system (Wiley, 1995).

HPLC purification was carried out on synthetic heptadienals to separate geometrical isomers. A silica column (Econosphere Silica, 5-μm particle size, 4.6 × 250 mm, Alltech, Deerfield, IL), treated with silver nitrate (Heath and Sonnet, 1980), was used for this purpose. The solvent was benzene:hexane (1:1). The pump was a Waters 515 (Milford, MA) (flow-rate 1 ml/min), and the detector was a Waters R401 differential refractometer.

^{1}H-NMR spectra ($CDCl_3$) were obtained on a Bruker Avance 400 MHz instrument (Billerica, MA).

Analysis of Commercial Heptadienals. Initial GC-MS analysis suggested that one male-specific compound was identical to a minor (ca. 5%) constituent of commercial (2*E*,4*E*)-2,4-heptadienal. A sample of this minor compound was purified by HPLC (250 μg per injection, 10 injections) for identification by NMR. The target compound was recognized in HPLC fractions by GC retention. In preparation for NMR, the combined sample in benzene/hexane was applied to a

small open-column of silica gel (0.5 × 3 cm), which was then eluted with pure hexane to remove the benzene and finally with redistilled diethyl ether to recover the aldehyde. The ether solution was evaporated under a gentle stream of nitrogen and $CDCl_3$ was added.

Quantitation. The amounts of (2*E*,4*Z*)-2,4-heptadienal (**1**) and (2*E*,4*Z*)-2,4-heptadien-1-ol (**2**) in the volatiles collections were quantified by GC-MS in selected ion monitoring (SIM) mode. Two ions were monitored for both **1** and **2** (*m*/*z* 81 and 110 for **1**; *m*/*z* 83 and 112 for **2**). The selected ions for the internal standard, 1-octanol, were *m*/*z* 70 and 84. Quantitation was based on ions *m*/*z*, 110, 112, and 84, whereas ions at *m*/*z* 81, 83, and 70 served as qualifiers (proper ion ratios would support compound identity and purity in the GC peaks). Serially diluted solutions of synthetic **1**, **2**, and 1-octanol (0.01 ng/μl −30 ng/μl) were analyzed by GC-MS in SIM mode to obtain linear calibration curves (log dose vs. log abundance), which served as the basis of the quantitation. In SIM mode, **1** and **2** were still easily detectable at concentrations as low as 10 pg/μl.

Chemicals. Compounds **1** and **2** were synthesized according to Petroski (2003). The purities were checked by GC and found to be >95%; impurities were other geometrical isomers. Samples of (2*E*,4*E*)-2,4-heptadienal and (2*E*,4*E*)-2,4-heptadien-1-ol were obtained from Bedoukian (Danbury, CT).

Field Lures. The lures for 2003 consisted of a 1:1 mixture of **1** and **2** (500 μg of each component). These were prepared by adding solutions (50 μg/μl) of synthetic **1** and **2** in methylene chloride to mineral oil (250 μl) in 1.5-ml glass vials. Vials were sealed with Teflon disks, held in place by caps with central holes for use with septa (SRI, Eatontown, NJ). The vials were wrapped in aluminum foil to afford UV protection. A wire, wrapped around at the base of the cap, was used to secure the lure to a trap. Prior to field deployment, the Teflon disks were pierced with a single pinhole to allow compound release from the headspace. Vials were kept upright in the field. The lures for 2004 were prepared as above except that **1** and **2** were used at 2.5 mg each per vial, and additional lures had just **1** alone (2.5 mg) or **2** alone (2.5 mg).

The emission rate of **1** and **2** was measured from a freshly prepared lure (1:1 mixture of **1** and **2**, 500 μg of each component) in an incubator. The vial was placed in a volatiles collector at 25°C with an air flow-rate of 100 ml/min. Volatiles were collected daily for a period of 5 d and the release rates of **1** and **2** were measured by GC-FID using 1-octanol as the quantitative internal standard. Compounds were released in a ratio of nearly 1:1 and at a rate of about 7 μg/day for each compound. Emission was consistent over 5 d. Less than 1% degradation of the aldehyde to the more stable all-*E* isomer was found.

Field Study. The experiments were carried out in saltcedar stands along the Humboldt River (40°01′N, 118°31′W) 17 Km southwest of Lovelock, NV, where a population of *D. elongata* has been established (DeLoach et al., 2004). Two types of experiments were carried out.

The first set was a pair-wise comparison of a 1:1 mixture of **1** and **2**, using five lures of 500 μg of each component, and unbaited controls, on July 15 and August 10, 2003, when newly emerged beetles were present. The 2003 experiments were carried out in saltcedar stands with relatively little defoliation. This experiment was repeated, using single lures of the same total dosage, on May 4, 2004, when the overwintered beetles were becoming active. The 2004 site had experienced complete defoliation late in 2003, and little foliage was present on these weakened trees during the pheromone trial. The numbers of replications per treatment for the three experiments were 20, 15, and 22, respectively.

The second experiment measured the effects of the individual compounds and was set up on May 5, 2004. Treatments were a 1:1 mixture of **1** and **2**, **1** alone, **2** alone (all at 2.5 mg per component), and an unbaited control. A randomized complete block design was used (four traps per block), and there were 20 replicates of each treatment.

Traps were placed in trees that were about 10 m apart and similar in size, foliage density, and in accessibility to beetles flying from downwind (the prevailing wind at the site was from the west). There were always other saltcedar trees in the vicinity of the trap trees and usually between them as well. Yellow sticky traps (15.5 $\times$ 30.5 cm, AgriSense, Pontypridd, UK) were attached to branches (oriented vertically) in the upper half of the trees, typically at a height of 2–4 m, and every effort was made to place traps at nearly the same height. The higher locations were accessed by bending branches to within reach. Foliage or defoliated branches surrounding the traps were removed as needed so that there was a clear "flight path" to the trap. The protective paper was removed only from one side of the trap, so that the sticky side was oriented downwind. The choice of which trap would receive a particular treatment was made randomly by coin flip. The dispensers were activated when they were attached to the traps by making a pin hole in the Teflon seal.

Traps were set out in mid afternoon, when flight activity usually began to increase. They were then examined at intervals of about 2 h, and counts of beetles were recorded. The next morning, the traps were removed. Final counts were made and the beetles trapped on July 16 were saved for determination of sex.

Statistics. Amounts of compounds **1** and **2** collected from the beetles and foliage controls were submitted to analysis of variance (ANOVA) using Statistix for Windows software (Analytical Software, 1998). The amount data were transformed to a log scale to stabilize variance. The composition data (amount of **1** as a percentage of **1** + **2**) were submitted to ANOVA without transformation. For the field test, counts of *D. elongata* adults on traps were transformed using log (X + 1) to stabilize variance. The data from the pair-wise experiments were analyzed by paired *t*-tests, and data from the randomized complete block design were submitted to analysis of variance (ANOVA) and means were compared using the least significant difference (LSD) test.

RESULTS

Volatiles Collections. By GC comparison of volatiles extracts, males of *D. elongata* feeding on saltcedar emitted two compounds, **1** and **2**, that were not immediately obvious in volatiles extracts from females feeding on saltcedar or saltcedar foliage alone (Figure 1). The unlabeled peaks in Figure 1 were primarily saltcedar volatiles, based on GC-MS comparisons to uninfested plant materials.

Spectral Analysis and Identification. Mass spectra of the two male-emitted compounds indicated molecular weights of 110 for **1** and 112 for **2**. A search of the mass spectral library indicated close matches with (2*E*,4*E*)-2,4-heptadienal and (2*E*,4*E*)-2,4-heptadien-1-ol for **1** and **2**, respectively. Commercial samples of the aldehyde and alcohol closely matched the mass spectra of **1** and **2**, but their retention times were slightly later than the natural materials, indicating that **1** and **2** were probably geometrical isomers of the all-*E* standards. Fortuitously, the commercial sample of (2*E*,4*E*)-2,4-heptadienal contained an impurity (approx. 5%) whose mass spectrum, polarity (elution from open silica column with 10% ether in hexane) and retention time matched that of the natural aldehyde **1** exactly.

The impurity was isolated by HPLC ($AgNO_3$ on silica) and analyzed by ^{1}H-NMR. The configurations of the double bonds in the aldehyde impurity were determined based on vicinal coupling constants for the olefinic protons. The

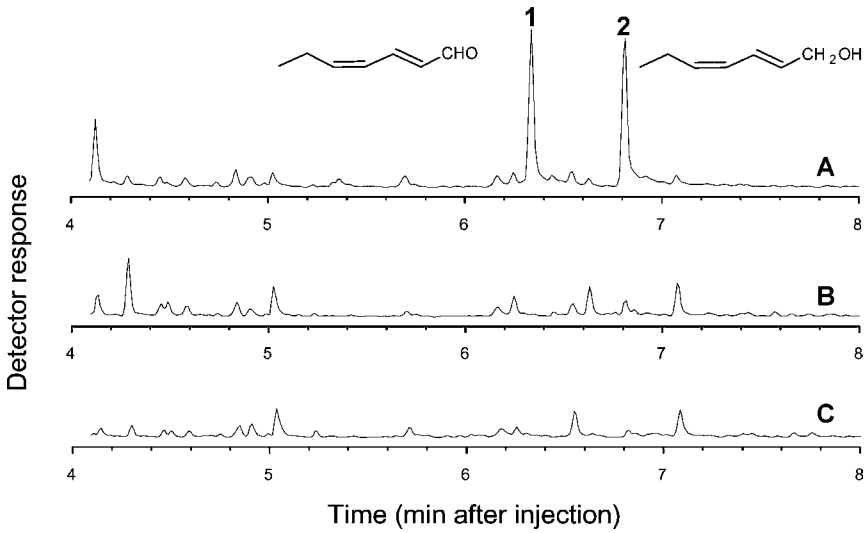

FIG. 1. Gas chromatographic profiles of volatiles collected from feeding *D. elongata*. A) males feeding on saltcedar foliage; B) females feeding on saltcedar foliage; C) saltcedar foliage control.

aldehydic proton (δ 9.61, 1H, d, $J_{1-2} = 8.0$, H-1) was coupled to one of these (δ 6.15, 1H, dd, $J_{1-2} = 8.0$, $J_{2-3} = 15.2$, H-2), and the magnitude of J_{2-3} established that the double bond in the 2 position had the *E* configuration (Williams and Fleming, 1980). The configuration of the second double bond was determined from the olefinic proton adjacent to the terminal ethyl group (δ 5.99, 1H, dt, $J_{4-5} = 10.7$, $J_{5-6} = 7.8$, H-5), and the magnitude of J_{4-5} indicated the *Z* configuration (Williams and Fleming, 1980). The other two olefinic protons (δ 7.44, 1H, dd, $J_{2-3} = 15.2$, $J_{3-4} = 11.5$, H-3; and δ 6.23, 1H, apparent br t, J_{3-4} and $J_{4-5} \cong 11$, H-4) were consistent with the above conclusions. The value of J_{3-4} also supported the conjugated diene system (Williams and Fleming, 1980). Thus, the configuration of the impurity and, therefore, of **1**, was 2*E*,4*Z*. Several of the olefinic proton signals were broadened by long-range allylic coupling. The ethyl group protons were observed (δ 2.39, 2H, apparent quin, J_{5-6} and $J_{6-7} \cong 7.5$, H-6; δ 1.11, 3H, t, $J_{6-7} = 7.5$, H-7).

The synthetic (2*E*,4*Z*)-2,4-heptadienal (**1**) was reduced with $LiAlH_4$ to give (2*E*,4*Z*)-2,4-heptadien-1-ol (**2**). The mass spectrum and retention time of this alcohol matched those of natural **2** exactly. Therefore, the two male-specific compounds were identified as (2*E*,4*Z*)-2,4-heptadienal (**1**) and (2*E*,4*Z*)-2,4-heptadien-1-ol (**2**) and subsequent synthesis of the two compounds further confirmed these identifications (Petroski, 2003).

A few of the extracts of male volatiles that showed relatively large amounts of **1** and **2** indicated the presence of at least two additional male-specific compounds (<1% of the total of **1** + **2**). These compounds eluted earlier from the GC columns than **1** and **2**, but they did not elicit antennal responses, and the small amounts available did not allow a more detailed examination.

Further examination of volatiles extracts from female beetles feeding on foliage and foliage controls by GC-MS did reveal trace amounts of **1** and **2**, based on both GC retention times (5.04 and 5.20 min, respectively) and mass spectra.

Electrophysiology. GC-EAD analysis of extracts of volatiles from male beetles feeding on saltcedar foliage showed that natural **1** and **2** were readily detected by the antennae of both sexes. Similar results were obtained for the synthetic materials (Figure 2). GC-EAD analysis supported the existence of **1** in volatiles from uninfested and female-infested saltcedar foliage. Antennal responses at the retention time for **1** (5.04 min) was observed in several cases in the initial analyses of female- and foliage-derived samples, and when such samples were combined and concentrated, antennal responses to **1** were observed more consistently. However, the presence of **2** (retention time, 5.20 min) in collections from foliage or feeding females was not verified by GC-EAD, presumably because the even lower amounts were below the antennal response threshold.

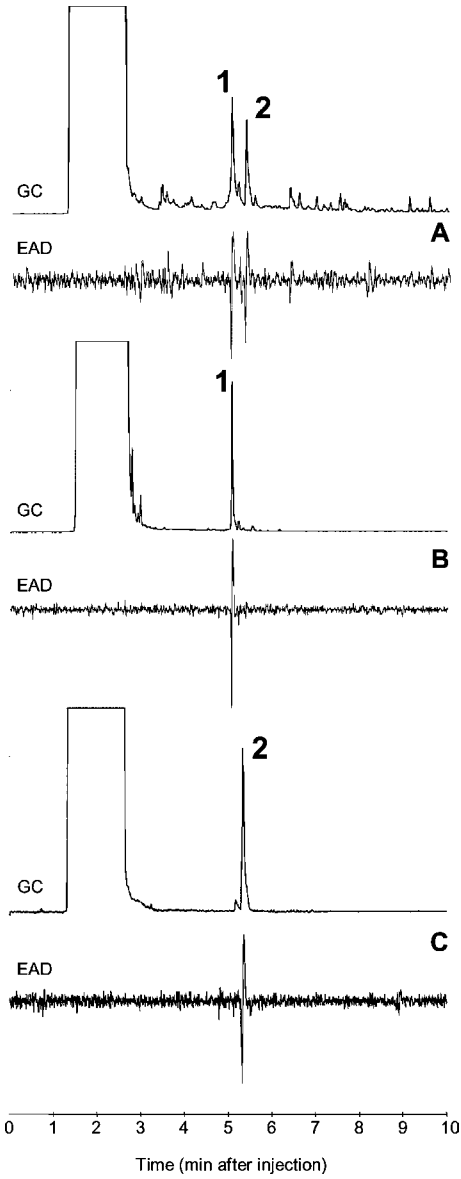

FIG. 2. Simultaneously recorded gas chromatogram (GC) and electroantennographic detection (EAD) of: A) female antenna responding to volatiles collected from male *D. elongata* feeding on saltcedar, including **1** and **2**; B) male antenna responding to synthetic **1**; C) male antenna responding to synthetic **2**.

Synthetic (2*E*,4*E*)-2,4-heptadienal and (2*E*,4*E*)-2,4-heptadien-1-ol were also detected by the antennae of both sexes (GC-EAD) when sufficient amounts of these isomers were injected (25 ng for each compound).

Emission Rates. Amounts of compounds **1** and **2** collected per day are shown in Table 1 for both sexes, and both densities (grouped and single) and for uninfested foliage. The amounts in the table are expressed both on a per-tube and a per-beetle basis, for comparison. Compounds **1** and **2** were obvious within days of placing new male beetles in the collection apparatus, and emission continued as long as the beetles lived (approx. 30 d) with fairly constant component ratios.

On a per-beetle basis, amounts of **1** and **2** from tubes with males were always significantly higher than from tubes with females (LSD, $P < 0.05$ level). Groups of males emitted significantly more of **1** and **2** compared to single males, but the trace amounts of **1** and **2** collected from tubes with grouped females did not differ significantly from those kept as individuals. The maximum amount of **1** and **2** collected from a single male was 1.57 μg/d.

Expressing amounts of **1** and **2** on a per-tube basis allowed the effect of foliage to be considered in comparisons, as well as beetles. The amounts of **1** and

TABLE 1. EMISSION OF (2*E*,4*Z*)-2,4-HEPTADIENAL (**1**) AND (2*E*,4*Z*)-2,4-HEPTADIEN-1-OL (**2**) BY SINGLE AND GROUPED (USUALLY 10) *D. elongata adults*

		Emission rate (ng/tube/day)			Emission rate (ng/beetle/day)	
Component	*N*	Mean[1]	Confidence interval[2]	**1** as % of (**1** + **2**)	Mean[1]	Confidence interval[2]
Male Beetles						
Grouped						
1 + **2**	31	860[a]	225–3320	61[a]	125[a]	46–330
Single						
1 + **2**	37	55[b]	8.7–350	60[a]	55[b]	8.7–350
Female Beetles						
Grouped						
1 + **2**	15	28[bc]	13–60	93[b]	3.1[c]	1.8–5.5
Single						
1 + **2**	14	7.1[d]	2.8–18	92[b]	7.1[c]	2.8–18
Foliage						
1 + **2**	5	8.1[cd]	4.4–15	84[b]	NA	NA
F statistics		37.2 ($df = 4,97$) $P < 0.001$		22.2 ($df = 4,97$) $P < 0.001$	33.2 ($df = 3,93$) $P < 0.001$	

[1]Untransformed means, means within a column followed by the same letter do not differ by LSD test, $P < 0.05$.
[2]Untranformed (log mean ± one standard deviation).

2 emitted by grouped males were dramatically higher than all other treatments, and the amounts from tubes with single males were higher than all treatments with females or foliage alone, except that the difference from grouped females was not significant. The amounts of **1** and **2** from tubes with females did not differ from those collected from the saltcedar foliage.

Considering composition, the percentage of **1** (relative to **1** plus **2**) was significantly lower in extracts from males than with the female or foliage-only extracts (Table 1). However, the percentage of **1** in extracts from females did not differ significantly from that found with uninfested foliage.

Field Study. Trapping experiments demonstrated the attractiveness of the two male-specific compounds to *D. elongata* in the field on four different dates with two different populations (Table 2). Significantly more adult *D. elongata* were trapped on traps baited with **1**, **2**, and a 1:1 mixture of **1** and **2** compared to the control traps.

However, compound **2** as a single component was as attractive as the 1:1 blend of the two components, whereas compound **1** as a single component was only weakly attractive (Table 2).

Male and female beetles were about equally attracted to the two component mixture. July 16, 2003 saw a total of 945 adults on the baited traps, with a male: female sex ratio of 1.1: 1; control traps caught a total of 351 beetles with a male: female sex ratio of 1.4: 1. Nearly all of the trapped beetles were caught between mid-afternoon and twilight, during the peak of flight activity. There was no evidence for beetles being caught between twilight and the final trap count the following morning.

TABLE 2. MEAN NUMBER OF *D. elongata* ADULTS PER STICKY TRAP BAITED WITH SYNTHETIC (2*E*,4*Z*)-2,4-HEPTADIENAL (**1**), (2*E*,4*Z*)-2,4-HEPTADIEN-1-OL (**2**), AND A 1:1 MIXTURE OF **1** AND **2**, IN LOVELOCK, NEVADA SALTCEDAR STANDS DURING 2003 AND 2004

	Mean number of beetles/trap			
	2003		2004	
Treatment	July 16	Aug. 11	May 5	May 6
1 + **2**	32.1**	13.7**	14.4**	76.7[a]
Control	7.9	4.0	1.4	5.3[c]
1	—	—	—	10.4[b]
2	—	—	—	65.5[a]
t statistics	4.63 (*df* = 19)	6.00 (*df* = 14)	5.58 (*df* = 21)	—
F statistics	—	—	—	69.79 (*df* = 3,57)
	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

DISCUSSION

Analysis of extracts of volatiles demonstrated that male *D. elongata* emitted two components, (2*E*,4*Z*)-2,4-heptadienal (**1**) and (2*E*,4*Z*)-2,4-heptadien-1-ol (**2**), which were sensed by antennae of both males and females.

However, in field trials, **2** was much more attractive than **1**, and **2** as a single component was as attractive as a 1:1 blend of **1** and **2**. Thus, the role of **1** as a component of the pheromone blend is not yet clear. Further trials with different blend ratios may reveal an additive or synergistic role for it in the blend. The synthetic pheromone attracted approximately equal numbers of males and females, indicating that it is an aggregation pheromone rather than a sex pheromone.

These compounds are quite different from previously described chrysomelid pheromones (see introduction), being smaller in size and simpler in structure (i.e., without branches or rings). Indeed, the compounds were overlooked in the early stages of this study because of their similarity to the well known, six-carbon "green leaf volatiles" (Visser and Avé, 1978).

An unusual result in this study is that compounds identical to the pheromone components could be found at trace levels in collections from feeding females and from the host plant. Of these two, the data suggest that the foliage is the actual source, rather than the females, because the collections from single, feeding females were essentially the same as collections from uninfested foliage. The collections from groups of females had significantly greater total amounts of the compounds than collections from single females, but on a per-beetle basis, the group collections did not differ significantly from the single beetles. A likely explanation is that the greater amount of feeding activity by groups of females led to a greater release of plant volatiles, including **1** and **2**. Further evidence for a foliar origin of the compounds in the collections from females is that a ratio of **1** and **2** was essentially the same as in the foliage, but was quite different from the collections from males.

It is not surprising that damaged foliage might emit aldehyde **1** because this compound is known to be an autoxidation product of the ubiquitous biochemical, linolenic acid (Frankel, 1998 and references therein). The more remarkable situation is that the amounts were so much higher when males were feeding on that foliage, and that the component ratios of **1** and **2** were so different from when males are absent, indicating that males exert a great deal of control over the emission of the compounds. Beyond the greater amounts and different ratios noted above, compound emission is closely linked to the physiology of the males and to their environment. For example, males induced to enter overwintering reproductive diapause by short daylength do not emit or induce the production of **1** and **2** (although they continue to feed), and a day/night emission rhythm has been observed in reproductive males. Furthermore, strains of beetles of various geographic origins also produce different, characteristic ratios of components **1**

and **2**. These (presently unpublished) examples of control over emission lead us to believe that males produce the pheromone within their bodies rather than inducing the host plant to emit them, despite the superficial similarity of the pheromone components to green leaf volatiles.

In the future, different doses and ratios of the two components should be tested in the field to determine whether alternative responses are possible. In addition, the blend/single component experiment should be repeated in areas with various degrees of defoliation, various population densities, and at various times of the year to further assess the effects of the single components and competition from natural sources of **1** and **2**. Also, volatiles collections in which components **1** and **2** were relatively abundant had additional, minor male-specific compounds, and the possible behavioral effects of these additional components remains to be evaluated. Finally, GC-EAD analysis indicated that some saltcedar derived compounds elicited antennal responses. Whether host odor is necessary for optimal field response will have to be studied, particularly as synergism between aggregation pheromones and host-related volatiles is known in the Chrysomelidae (Metcalf and Metcalf, 1992; Pivnick et al., 1992).

A pheromone-based monitoring system will be useful for studying several population attributes of *D. elongata*. The field results demonstrated the potential of using the synthetic pheromone for monitoring field populations of *D. elongata*, with the added benefit that both sexes can be monitored.

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